4:30

765-3 Superoxide Dismutase Reduces Superoxide Anion Levels in Balloon-injured Porcine Coronary Arteries

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Superoxide anion (O_2-), a moiety that rapidly inactivates nitric oxide, is present in elevated concentrations in the vessel wall following balloon vascular injury. Recent data suggest that O_2- is involved in the abnormal endothelium-dependent vasomotor responses of the regenerated endothelium.

To investigate the hypothesis that polyethyleneglycol superoxide dismutase (PEG-SOD) can effectively scavenge O_2- in the vessel wall, ten pigs were subjected to balloon injury of the left anterior descending or left circumflex coronary artery, then maintained on normal lab diets for 28 days. On day 29 five pigs (RX group) began receiving daily intravenous infusions of PEG-SOD (12,000 U/kg day 1, then 6,000 U/kg days 2–5) while five pigs received no PEG-SOD therapy (NO RX group). All animals were sacrificed on day 33. O_2- generation was assessed by lucigenin-amplified chemiluminescence in segments of injured and uninjured coronary arteries. Uninjured/NO RX segments ($n = 7$) and uninjured/RX segments ($n = 7$) had similar O_2- levels (2728 ± 457 and 3540 ± 543 counts per minute/mg tissue dry weight, respectively, p = NS). Injured/NO RX segments ($n = 5$) had significantly elevated O_2- production (7040 ± 1608 cpm/mg, $p < 0.05$ vs baseline). PEG-SOD therapy in injured/RX segments ($n = 7$) dramatically restored O_2- levels to normal (3167 ± 681 cpm/mg, p = NS vs uninjured, $p < 0.05$ vs injured/NO RX).

Thus, PEG-SOD therapy effectively reduces O_2- levels in the vessel wall following balloon injury. Prevention of nitric oxide degradation via this mechanism may have important implications for modulation of coronary vascular tone following balloon injury.

4:45

765-4 Adhesiveness of Mononuclear Cells is Increased in Hypercholesterolemic Humans, and Reduced by the NO Precursor

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Enhanced interaction of mononuclear cells with the endothelium is the first observable event in the development of atherosclerosis. Hypercholesterolemia reduces vascular nitric oxide activity. This dysfunction may promote endothelial monocyte interaction, as NO is a potent inhibitor of adhesion. We have previously shown that dietary L-arginine (Arg) supplementation in hypercholesterolemic rabbits restores NO activity and inhibits monocyte-endothelial cell interaction, in association with a reduction in atherogenesis. Accordingly we developed a functional binding assay to assess the adhesiveness of human mononuclear cells, so as to determine the effects of hypercholesterolemia and L-arginine therapy. We found a 50% increase in adhesion of mononuclear cells (MNC) from hypercholesterolemic (HC) subjects to cultured endothelial cells *ex vivo* compared to a normocholesterolemic control population ($p < 0.0001$, $n = 20$). Increased MNC adhesion was reversed to normal levels by preincubation of the MNC suspension with 10^{-5} M sodium nitroprusside (164.4 ± 8.7% vs 97.5 ± 6.9%, $p < 0.0005$, $n = 7$), while L-nitroarginine and Arg did not have an effect *in vitro*. In a double-blinded placebo-controlled study, oral Arg (8.4 g/day) was administered to 7 HC subjects. Over a course of two weeks, this treatment abolished the increased adhesion (158.5 ± 10.9% vs 103.5 ± 4.9% vs 100 ± 5.2%, HC vs Arg vs Control, $p < 0.005$), while MNC adhesion remained significantly elevated in placebo-treated subjects. *Conclusion:* The adhesiveness of human mononuclear cells is increased by hypercholesterolemia. The increase in adhesiveness is reversed *in vitro* by NO donors, and reversed *in vivo* by treatment with the NO precursor L-arginine.

5:00

765-5 Platelet Inhibitory Effect of Endothelium-derived Relaxing Factor in the Human Coronary Circulation

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Introduction: Endothelium-derived relaxing factor (EDRF) inhibits platelet activation by increasing platelet cyclic GMP content. In the human coronary circulation the vasodilator effect of EDRF released abluminally from the endothelium is well described, but the effects of luminal release of EDRF on platelets is unknown. Furthermore, whether decreased vasodilator responses to agents that stimulate EDRF release are also associated with an abnormal platelet response is unknown.

Methods and Results: We therefore studied the effects of intracoronary acetylcholine (ACh), which stimulates the endothelial release of EDRF, on blood flow and platelet cGMP content in 14 patients with agiographically normal coronary arteries undergoing cardiac catheterization. Seven patients received sodium nitroprusside (SNP) infusions. Blood flow was derived from Doppler flow velocity, and diameter was measured by quantitative angiography. Simultaneous samples were drawn from the great cardiac vein for measurement of platelet cGMP content by radioimmunoassay. During ACh infusion (30 μg/min) there was a transient increase in both coronary flow 138 ± 25% (mean ± SEM, $p < 0.01$) and platelet cGMP content (34 ± 13%, $p < 0.01$), that returned to baseline (both $p < 0.01$). Epicardial diameter responses to ACh were heterogenous: seven patients showed constriction (−7 ± 2%) and seven showed dilatation (11 ± 2%). Changes in platelet cGMP content and epicardial diameter paralleled each other: in patients with dilatation, ACh increased cGMP content significantly (54 ± 22%, $p < 0.02$), whereas in those with constriction the change in cGMP was insignificant (14 ± 8%, p = NS). During SNP infusion (40 μg/min) flow increased by 127 ± 23% and cGMP content by 226 ± 55% ($p < 0.02$).

Conclusions: 1) luminal release of EDRF in the human coronary circulation causes an increase in platelet cGMP content; 2) this effect is less evident in patients with impaired endothelial-mediated vasodilation. Failure of this platelet inhibitory effect in patients with atherosclerosis and endothelial dysfunction may contribute to their increased susceptibility to thrombotic vascular events.

5:15

765-6 Thromboxane Mediates Impaired Coronary Microvascular Responses to Metabolic Stimulation in Diabetes

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Our laboratory has previously demonstrated that nitric oxide (NO) mediates coronary arteriolar dilation to increased myocardial oxygen consumption (MVO_2). NO mediated dilation has been shown to be attenuated in diabetes (DM), and endoperoxide antagonists restore this dilation to normal. We, therefore, studied the effect of DM on metabolically mediated dilation of coronary arterioles. We further tested the effect of SQ29548 (SQ; thromboxane A2 and prostaglandin H2 receptor antagonist) on coronary arteriolar dilatory responses to increased MVO_2 in DM. We measured changes in epicardial arteriolar diameters in 5 DM (1 week alloxan, 60 mg/kg, iv), 4 DM after administration of SQ (2 mg/kg, iv), and 6 normal dogs during increased MVO_2, using dobutamine (DOB; 10μg/kg/min, iv) with rapid atrial pacing (RAP; 288 ± 4 bpm). Diameters were measured using intravital microscopy in anesthetized dogs with stroboscopic epi-illumination coupled to the cardiac cycle. Mean aortic pressure and blood gases were monitored and kept constant throughout the protocol. *Results:*

	Control		DOB		DOB + RAP	
	MVO_2	diameter (μm)	MVO_2	%Δ in diameter	MVO_2	%Δ in diameter
Normal	11 ± 1	60 ± 5	23 ± 2	24 ± 4	39 ± 7	43 ± 6
DM	21 ± 7	78 ± 6	36 ± 13	17 ± 5	40 ± 13	14 ± 3*
DM + SQ	16 ± 3	82 ± 9	22 ± 1	20 ± 3	41 ± 5	37 ± 7

mean ± SEM, * $p < 0.05$ vs. Normal. MVO_2 is ml/min/100 g

In DM animals, during DOB + RAP, arterial-venous oxygen difference increased as flow decreased, thus keeping MVO_2 similar. Thus, coronary arterioles in diabetes demonstrate attenuated microvascular dilation to increased myocardial oxygen consumption, and vasoconstrictor endoperoxides play a key role in this impaired response.

766 Molecular Basis of Cardiovascular Disease: From Genotype to Phenotype

Tuesday, March 21, 1995, 4:00 p.m.–5:30 p.m.
Ernest N. Morial Convention Center, Room 64

4:00

766-1 Angiotensin Converting Enzyme Gene Polymorphism in Acute Myocardial Infarction Patients

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Background: A polymorphic marker of the ACE gene has been related to